Wound Healing

Introduction

Wound Healing explores what happens microscopically within a wound to produce a healed macro result. The details contained are relevant to the boards. But more importantly, **normal wound healing** does a lot of what makes a cancer malignant. The normal, healthy response of forming a scar from a wound involves migration from a blood vessel, angiogenesis, destruction of the local extracellular matrix, and proliferation of cells. We will use what we learn in this lesson again as we begin to discuss cancer. In each phase, pay close attention to the primary activity, the cells that are active, and the reasons for delayed wound healing. This process is also seen in tissue necrosis throughout the body and will be used to explain healing, repair, and malignant transformation throughout the Organ Systems. The images are snapshots from the accompanying animation video in the treasure chest on the lesson page.

Phases of Wound Healing

Phase 0: Hemostasis, 0–30 minutes. This is not traditionally taught as a separate phase and is often wrapped into the inflammatory phase. Immediately after injury, as long as there is no deficiency in clotting, the blood will clot. Clot formation involves both platelets (primary hemostasis) and clotting factors (secondary hemostasis). The details of platelet clotting and clotting factors are discussed in Hematology.

The bleeding wound **clots**, thereby achieving **hemostasis** and **preventing dehydration**. The epithelium should be doing this. But after it's been opened by the injury and before new epithelium can fill the gap, the thrombus acts as a temporizing agent. The clot in the wound will act as scaffolding for inflammatory cells (neutrophils, then macrophages) and fibroblasts to adhere to. The clot on the skin is called a scab and will provide protection from invading microbes and dehydration.

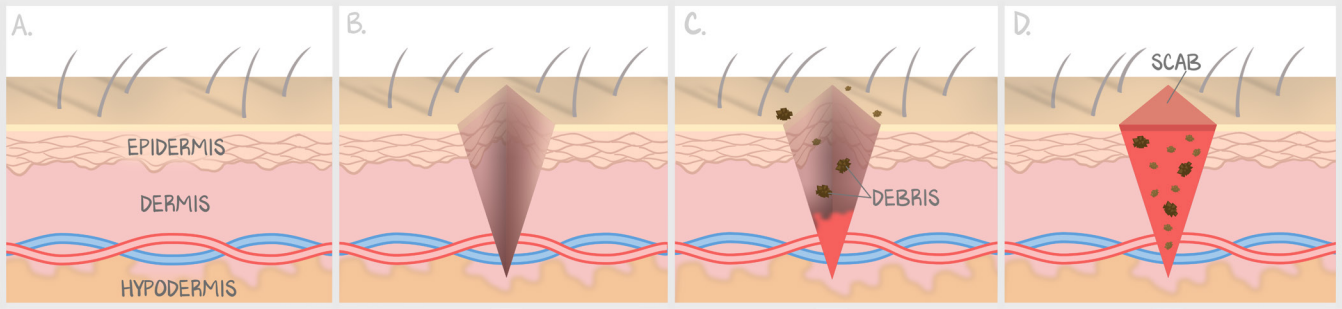

Figure 4.1: Injury and Phase 0
(a) Intact stylized epithelium on top, a vascular dermis below, and the hypodermis, which will not be engaged in this image series. (b) A wound is inflicted through the dermis, vessels are cut and bleed. The cut is clean (sharp edges) and small. (c) Debris has penetrated the cut and into the bleeding. (d) A clot forms at the epithelial edge, preventing further contamination and inducing homeostasis.

Phase 1: Inflammatory Phase, 0–3 days. As everywhere in an inflammatory reaction, **neutrophils** arrive first and begin sweeping the clot of debris and pathogens. **Digestive enzymes** are secreted to kill off and degrade **bacteria**, foreign particles, and cellular debris. Neutrophils also release **cytokines** to **summon macrophages**. Circulating monocytes receive the signal from neutrophils and enter the tissue, where they become macrophages. In the first 24 hours, **neutrophils** do the work. After 24 hours, the neutrophils have done their work and they "tag in" the macrophages. Monocytes are macrophages, monocytes in blood, macrophages in tissue.

Macrophages will remain through the proliferative phase into the maturation phase. Initially, they are present for **phagocytosis**, removing any foreign particles and ensuring that the defenseless fibroblasts have a clean space on which to get working. Macrophages also release **fibroblast growth factor**, which is a way of telling the fibroblasts it's safe, getting them to move in.

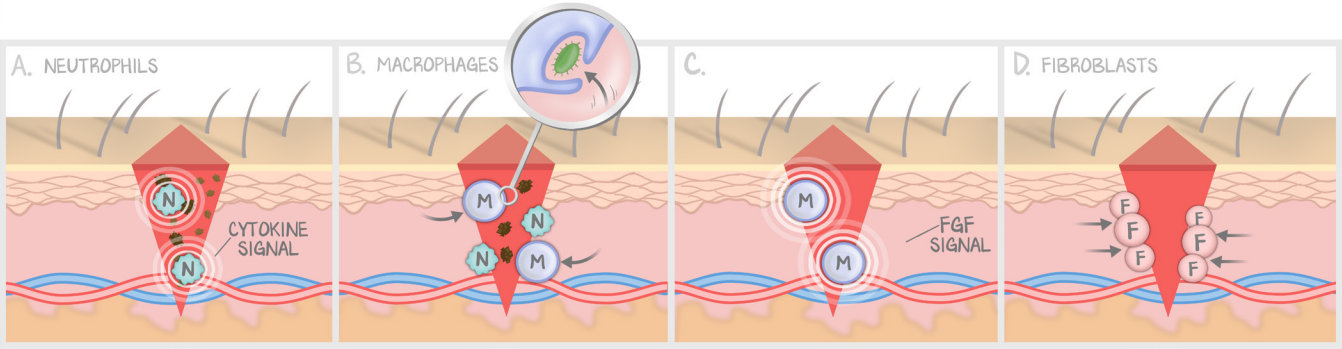

Figure 4.2: Inflammatory Phase
(a) The first responders are neutrophils, which phagocytose the bacteria and acellular debris. (b) Neutrophils recruit macrophages—stronger phagocytes than neutrophils. (c) After the debris and bacteria are eliminated, macrophages signal the all-clear for fibroblasts via FGF. (d) The fibroblasts arrive.

See the neutrophils as the forward scouts. They arrive first and use sniper fire to pick off key officers in the ranks of the foreign invaders of the wound. The neutrophils signal the macrophages to move in. Macrophages are the heavy armor, the stormtroopers. They come in with force and finish off the remaining troublemakers. Having secured the position, they remain to patrol it. The wound itself and subsequent fighting against the invaders has left the territory riddled with craters, the land decimated. Now with the invaders eliminated, macrophages signal the civilians—the fibroblasts—to come in and repair the damage.

Infection is a major source of delayed wound healing or failure of wound healing. The fibroblasts are civilians—construction workers, farmers, and engineers—who need a clean space to work. They can't fight for themselves. The new infrastructure is fragile. Any persistent (or new) invader can sabotage their work. Neutrophils and macrophages should keep the invaders at bay. Failure to do so could jeopardize the fibroblasts' work.

Phase 2: Proliferative Phase, 3–7 Days. With the "all-clear" signal from the macrophages (fibroblast growth factor), the **fibroblasts migrate in**. This migration is simultaneous with that of several other cell types, and together the new cells and materials make up the granulation tissue. **Granulation tissue** forms along the edges of the wound first, crawling down from the epithelium. Once the granulation tissue reaches the base of the wound, it begins to build "up." Using the clot as a scaffold, all cells work simultaneously. **Fibroblasts** lay down **collagen**, **myofibroblasts contract** the wound, **endothelial** cells generate new **blood vessels**, and **macrophages** clear the **scaffolding** when it isn't need anymore.

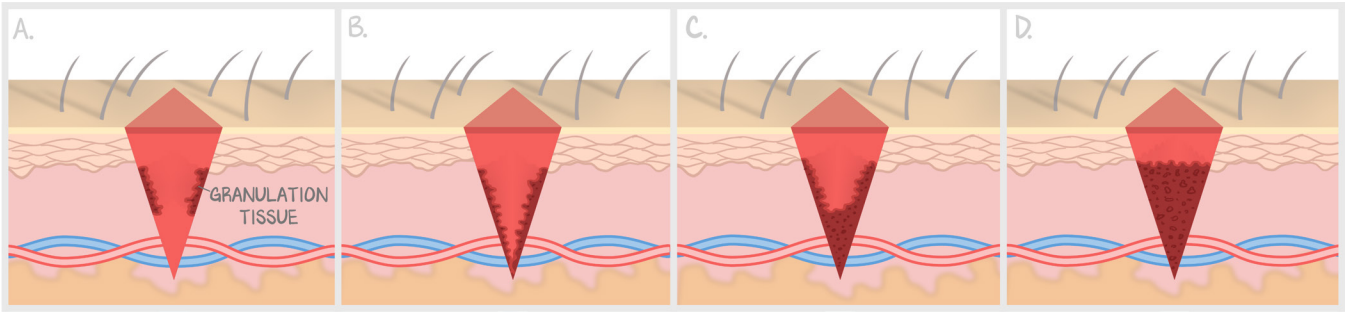

Figure 4.3: Proliferative Phase—Granulation Tissue
(a) Granulation tissue works its way down the edges, (b) making contact at the base, (c) then growing up, filling the wound from the base towards the epithelium and using the clot as a scaffold, until (d) the granulation tissue reaches the epithelial layer.

Fibroblasts are the real workhorses of the operation. They secrete **fibronectin**, which acts both as an **opsonizing agent** (to help the macrophages with phagocytosis) and a **chemotaxis** agent for more fibroblasts. Fibroblasts also secrete **VEGF**, which, together with **platelet-derived growth factor** (PDGF) and **TGF-β**, induces **angiogenesis**. Meanwhile, the fibroblasts are layering **collagen type III** across the wound, parallel to the basement membrane. This layering of collagen **lacks tensile strength**, yet this orientation is needed to contract the wound, bringing the edges closer together. Collagen I is a stronger orientation will replace this collagen during the maturation phase.

Myofibroblasts are a mix between smooth muscle and fibroblasts. They are responsible for **contraction of the wound edges**. They are "fibroblasts" in that they use collagen—true fibroblasts lay down collagen, and myofibroblasts use that collagen. They are "myo" because of how they use it—contracting like smooth muscle, they use the collagen to bring the edges of the wound together. Fibroblasts lay down collagen type III so that the myofibroblasts can use it to bring the would edges together. As the granulation tissue heals and scars from the bottom up, the granulation tissue pulls in on all sides to close the wound.

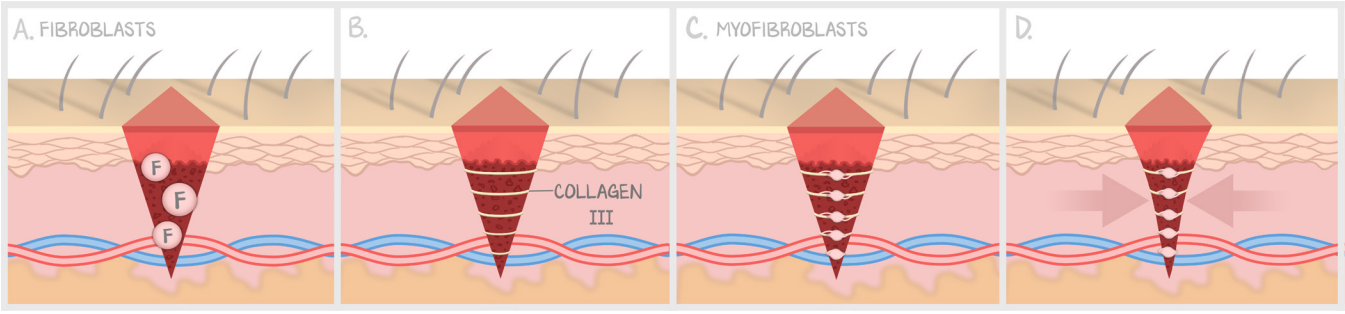

Figure 4.4: Proliferative Phase—Fibroblasts
(a) Fibroblasts lay down collagen. (b) First, they lay collagen across the wound (c) so that the myofibroblasts can contract along the collagen III, (d) bringing the edges of the wound closer together. To supply this activity, blood vessels are simultaneously created.

These cells are **highly metabolically active** and so require an **abundant supply of glucose and oxygen**. To do that, **angiogenesis** promotes a rich irrigation network within the granulation tissue. **Endothelial cells** are induced to **proliferate and migrate**, forming **new blood vessels** from existing ones. Stable (in G_0) endothelial cells are both pushed back into the cell cycle to proliferate and are induced to migrate from the blood vessel into the granulation tissue. The proliferative granulation tissue is primarily fibroblasts laying collagen surrounded by a dense network of capillaries. This is why even healthy wounds are **friable bleeders**—the clot is being dissolved, replaced with collagen and rich vasculature.

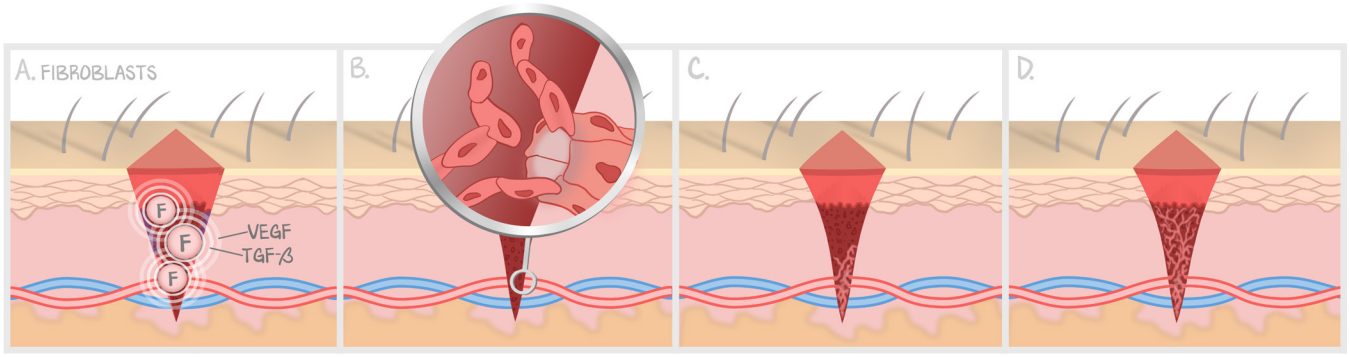

Figure 4.5: Proliferative Phase—Angiogenesis
(a) Fibroblasts secrete angiogenesis chemokines, VEGF, and TGF-β. (b) New blood vessels come from quiescent endogenous blood vessels, proliferating from already existing vessels. (c) Vessels start near the large vessels and (d) migrate through the granulation tissue.

As the granulation tissue reaches the basement membrane and blood supply is restored locally by angiogenesis, **keratinocytes** (the epithelium of skin) close the gap around the clot. **Epithelial cells** proliferate toward one another, and use **E-cadherins** as a signal to stop proliferating once they bump into one another. The scab is removed and the patient sees intact skin. However, in the dermis beneath, fibroblasts continue to work.

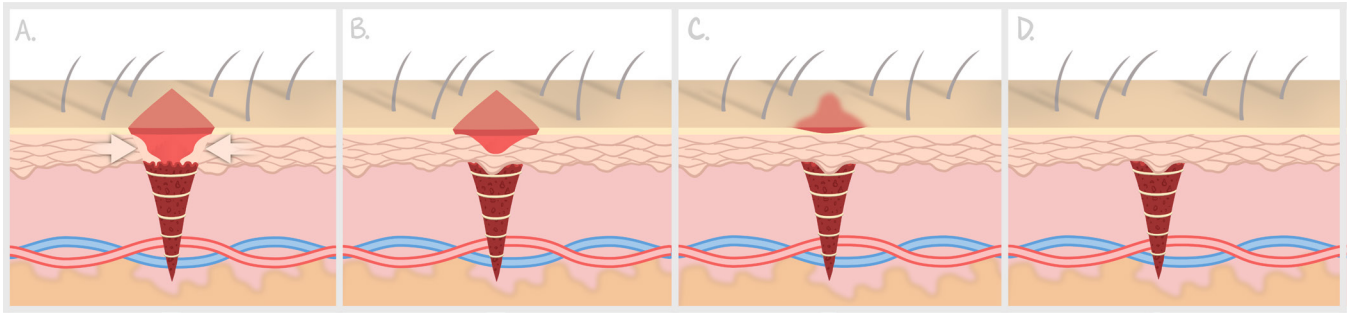

Figure 4.6: Proliferative Phase—Epithelial Cells
(a) As the granulation tissue reaches the epithelial layer, it is induced to proliferate, (b) growing in from the sides atop the granulation tissue as a scaffold until that basal layer meets in the middle (c) then grows up from the base until (d) the epithelium is restored.

This proliferation phase is a very metabolically active process. The massive degree of angiogenesis shows that wound healing requires an abundance of blood flow. The fibroblasts require nutrients and removal of waste products. Macrophages require oxygen to fight pathogens. Delayed or failed wound healing may occur as a product of **compromised vascular supply**. That is, despite having angiogenesis within the wound, if the blood can't get to the wound, the wound won't have the glucose and oxygen required to heal. A compromised vascular supply might be in the form of **microvascular disease**, such as in diabetes mellitus. For this, there is little that can be done. While diabetic control and anti-platelets can prevent progression, the poor perfusion cannot be surgically corrected. **Macrovascular disease**, such as peripheral arterial disease, can be treated with **stenting or bypass**. Finally, if in addition to poor vasculature there is infection, prolonged antibiotics can be attempted, but often to save the patient the limb is **amputated**.

Poor nutrition (vitamin C) has been implicated here as well. There is no strong evidence that vitamin C improves wound healing, though the risk, safety, and tolerability of adding vitamin C makes it a common recipe in wound-healing practices. Vitamin C is thought to promote macrophages. **Poor nutrition** (low protein) compromises wound healing. It's why surgeons will not perform an elective procedure on a patient with profoundly low albumin. Albumin is a marker of protein calorie nutrition. Increasing proteinaceous calories will ensure adequate supply of amino acids to the healing wound, and general protein levels can be monitored by tracking the albumin, prealbumin, and CRP. Best to restore the nutritional status of a patient before surgery, if able.

Phase 3: Maturation, weeks to years. As the granulation tissue rises from the base of the wound, the clot has been degraded and removed by macrophages. By the maturation phase, the clot is gone. What is left in its place is collagen III layered to provide contraction of the wound and an abundance of blood vessels that fueled the activity. Over the next several months, there will need to be a substantial rearranging of collagen and blood vessels to transition the granulation tissue to the eventual scar.

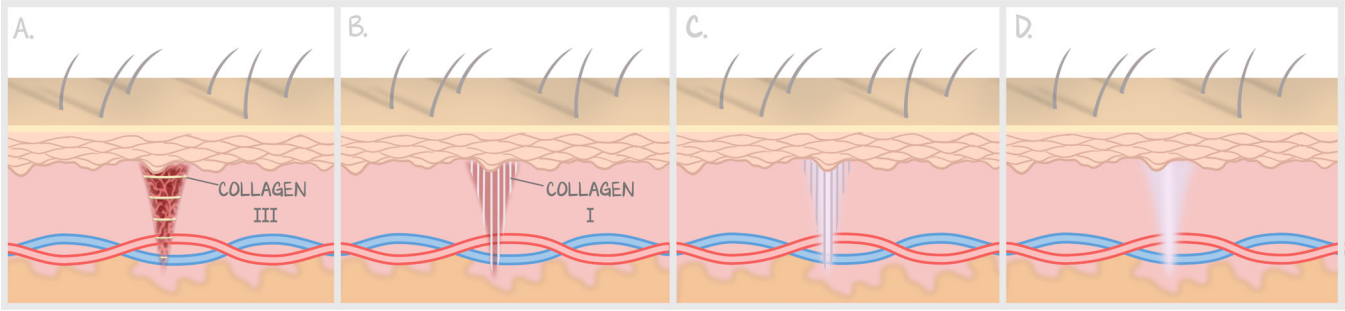

Figure 4.7: Maturation Phase
(a) Collagen III oriented perpendicular to the wound changes to (b) collagen I oriented perpendicular the epithelium, (c) angiogenesis reverses, and (d) the final acellular scar made of collagen remains.

Now that the wound is approximated (the wound edges have contracted together), **fibroblasts** switch collagen type and orientation to provide **tensile strength** rather than contractile force. Even with everything going correctly, a scar will only ever achieve a maximum of **80% of the original tensile strength** of intact tissue. The process of switching from contractile collagen to tensile collagen is mediated by **collagenase**. Collagenase requires **zinc** as a cofactor. Collagenase breaks down collagen III in its contractile arrangement and replaces it with **collagen I in a tensile positioning** (perpendicular to the basement membrane). The metabolic activity of the maturation phase is substantially less than that of the proliferative phase, which means that all of that angiogenesis and all those new blood vessels that formed in the wound, are no longer needed in the scar. Therefore, **angiogenesis reverses**, restoring the original blood vessel from which this network was spawned.

In the end, the dermis beneath the epithelium is now an **acellular scar** full of **collagen I** without any blood vessels.

Delayed wound healing of this phase is likely a product of **malnutrition** in the way of **zinc cofactor**. If delayed healing is identified, oral zinc may be added, though it is rarely added to a long-term regimen prophylactically without first identifying delayed wound healing.

VBAC and Tensile Strengths of Scars

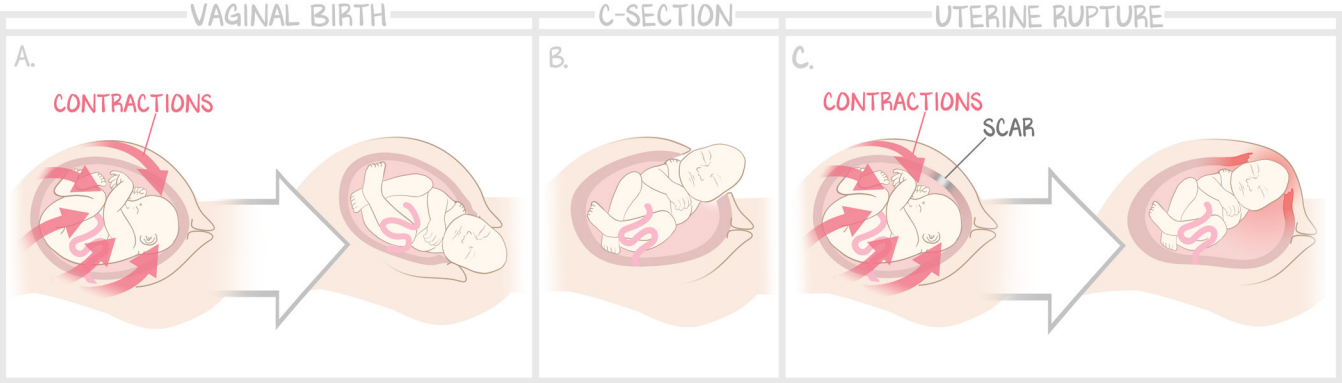

Figure 4.8: VBAC
(a) A normal vaginal delivery showing the uterus contracting to push the baby through the vaginal canal. (b) Cesarean delivery gets baby out through the abdominal wall, but leaves a scar. (c) Scar is weaker than the original tissue, so there is a risk of uterine rupture, where baby is delivered into the peritoneal cavity.

A scar is the weakest area and most likely to rupture under tension. This is why vaginal birth after cesarean (VBAC) is such a big concern. The scarred uterus has **at most** 80% tensile strength at the scar, and the more scar there is, the worse the tensile strength. Uterine rupture has a much higher chance of occurring during VBAC because of the scar—the healthy uterus contracts as hard as it wants, expecting there to be no scar, and the scar cannot tolerate the force of uterine contraction. The intact uterus, without scar, is equally strong all the way around.

Primary vs. Secondary Intention

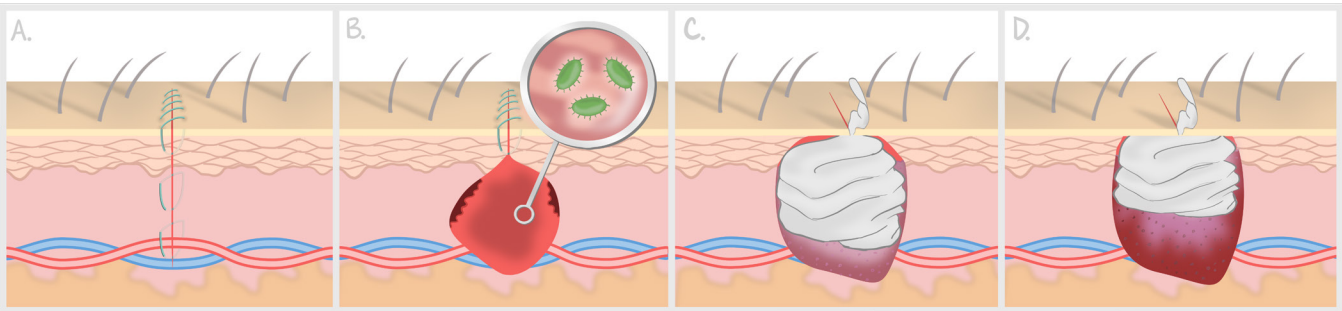

Figure 4.9: Primary vs. Secondary Intention
(a) Surgical incisions are closed by primary intention, approximating the edges and suturing them closed. This promotes minimal scar formation, and limits scar formation. It is preferred for elective surgical procedures. (b) If a large wound is sutured, it simply breeds infection. (c) Initially, lots of packing is required to heal by secondary intention. (d) Over time, granulation tissue fills in the wound and less packing is required.

Healing by **primary intention** describes the condition where the wound is caused by a **clean cut** and the edges **approximate one another** (they are close together). Primary intention is what healing looks like with a paper cut, or, medically, when a surgeon opens skin. The wound is caused by a sharp edge, and the wound opens in two directions, as if split in a plane. A clean, surgical incision, even if deep (such as to access the peritoneum), can be closed up well. If any wound is left to heal, it will create a scar. The closer the edges are approximated, the cleaner the cut, and the smaller it is, the **less scar there will be**. With primary intention, the **application of a suture** can facilitate the scar's appearance, size, and

strength. This reduces the amount of space that has to be managed by granulation tissue and improves the **cosmetic and tensile** strength of a wound.

Healing by **secondary intention** describes the condition where there is just too much space, the wound too large, or pathology removed too large that the edges **cannot be approximated**. Therefore, a substantial gap must be filled in by granulation. Granulation tissue still starts at the edge of the wound, migrates down to the base, then migrates across the base from both sides until it meets in the middle and "builds up." The problem with secondary intention is that this wound is not approximated, there is no clot as scaffolding, and so the granulation tissue doesn't know what to do or where to go. And this will take a LONG time to fully heal. Because there is no clot to act as scaffolding, scaffolding needs to be provided on which the granulation tissue can grow. This is **packing**. Each day the packing is removed and replaced with new. A little less packing is required each time as the granulation tissue continues to use it as the scaffolding. The packing acts as the clot did in primary intention. Very purposefully, however, we **never suture closed** a wound healing by secondary intention. It will lack the scaffolding and blood flow, serving only as a warm, moist environment to fester.

Abnormal Healing

Abnormal wound healing and delayed wound healing were discussed in tandem. Impaired clotting, infection, vascular insufficiency, and nutritional deficiencies (zinc yes, vitamin C maybe) can cause delayed wound healing.

Hypertrophic scars are ugly scars—people don't like them. But they are **not pathological** and carry **no increased risk for cancer**. Hypertrophic scars are **collagen III**, are always contained in the area of the original scar, and the collagen is in **parallel order**.

Keloids are pathological. They **overgrow** and are a **risk for malignant transformation**. African Americans are at higher risk of keloid formation (anyone can get a keloid, but the test will tell you African American). The keloid, if removed, will **recur**. It is a **disorganized** jumble of **collagen I and III**.

DELAYED HEALING	
Phase 0	Bleeding disorder
Phase 1	Infection
Phase 2 Phase 2	Microvascular (DM) Macrovascular stent or bypass
Phase 3	Zinc deficiency

HYPERTROPHIC SCAR	KELOID
Collagen III, parallel	Collagen I and III, disorganized
Never outside original margins	Always outside original margins
Does not recur	Does recur
No risk for malignancy	Risk for malignancy
No racial predilection	African Americans

Table 4.1: Abnormal Wound Healing
A summary table of the causes of delayed wound healing by phase, then a comparison of hypertrophic scar and keloids.

FACTOR	WHAT IT DOES
Platelet-derived growth factor (PDGF)	Secreted by activated platelets (phase 0) Vascular remodeling, smooth muscle migration Fibroblast growth factor
Fibroblast growth factor (FGF)	From fibroblasts, stimulates angiogenesis
EGF	Tyrosine kinase growth signal (trophic factor)
TGF-β	Angiogenesis
Metalloproteinases	Tissue remodeling
VEGF	Angiogenesis
Fibronectin	Opsonization, fibroblast summoning

Table 4.2: Active Chemokines in Wound Healing and Their Effects
A list of proteins, chemokines, and mediators lined up with what they do and from which cell they come from.

PHASE	PLAYERS	MECHANISM	WHAT HAPPENS
Phase 0 Hemostasis 0–30 minutes	Platelets	1° hemostasis: Plt plug 2° hemostasis: Fibrin clot	Hemostasis: Scab on top, clot under Prevents dehydration Acts as scaffolding
Phase 1 Inflammatory 0–3 days	Neutrophils	Digestive enzymes Cytokines	Degrade debris, bacteria, etc. Summon macrophages
	Macrophages	Phagocytosis	Phagocytosis clears, cleans slate for phase 2
Phase 2 Proliferative 3–7 days	Fibroblasts	Fibronectin Collagen III VEGF	Opsonization, chemotaxis, fibroblasts Collagen III fills wound “up”; organized to contract
	Myofibroblasts	“Smooth muscle” like	Contraction of the wound
	Endothelium	PDGF, VEGF, TGF-β	Angiogenesis to supply granulation
	Epithelium	E-cadherin, β-catenin	Seals epithelium, grows “in”
	Macrophages	FGF, phagocytosis	Promotes fibroblasts, clears PMNs, clears clot
Phase 3 Maturation 1 week–years	Fibroblasts	Collagenase (requires Zn)	Collagen III to collagen I Contraction to tensile orientation (80% max) Angiogenesis reverses

Table 4.3: Summary Table
A running account of the phases, the players, and what is happening in each phase of wound healing.